

SUGAR COMPOSITION, TOTAL NITROGEN AND ACCUMULATION OF C-¹⁴ ASSIMILATES IN FLORAL NECTARIES OF *PROTEA* SPECIES

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ABSTRACT

Sugar composition of nectar of six species of *Protea* was analysed by gas-liquid chromatography and consisted of sucrose, fructose and glucose in varying proportions. Total nitrogen content of nectar of *P. repens* and *P. neriifolia* was very low. Evidence is presented that nectar produced by inflorescences (e.g. *P. repens* and *P. neriifolia*) which are bird-pollinated are dominated by fructose and glucose whereas nectar of putatively rodent-pollinated inflorescences (e.g. *P. tenax*, *P. humiflora* and *P. denticulata*) contain mixtures of sucrose, fructose and glucose. By exposing leaves of flowering shoots of *P. neriifolia* to ¹⁴CO₂, studies revealed that bracts accumulate C-¹⁴ assimilates and translocation of compounds from the leaves to the floral nectaries was not affected by night and day incubation periods.

UITTREKSEL

DIE SUIKER SAMESTELLING, TOTALE STIKSTOF EN 'N OPEENHOOPING VAN C-¹⁴ ASSIMILATE IN DIE BLOMNEKTARKLIERE VAN *PROTEA* SOORTE

Die suiker samestelling van nektar van ses *Protea* soorte is met gasvloeistof-kromatografie analiseer en dit het bestaan uit sukrose, fruktose en glukose in wisselende verhoudings. Die totale stikstof inhoud van die nektar van *P. repens* en *P. neriifolia* was baie laag. Bewys word gelever dat die nektar wat deur bloeiwyses (bv. *P. repens* en *P. neriifolia*) wat deur voëls bestuif word, meer fruktose en glukose bevat, terwyl die nektar van die bloeiwyses van gewaande knaagdier bestuifde soorte (bv. *P. tenax*, *P. humiflora* en *P. denticulata*) mengsels van sukrose, fruktose en glukose bevat. Deur blare van blom-mende lote van *P. neriifolia* aan ¹⁴CO₂ bloot te stel, het dit geblyk dat die skutblare die C-¹⁴ ophoop en dat die beweging van verbindings van die blare na die blomnektarkliere nie beïnvloed was deur die dag en nag broeiperiodes nie.

INTRODUCTION

The genus *Protea* forms a conspicuous element of the fynbos vegetation of the south-western Cape (Taylor, 1978). Many *Protea* species require cross-pollination (Horn, 1962) and some are classified as either bird-pollinating or rodent-pollinating species (Rourke and Wiens, 1977), although other pollination systems probably occur in the genus. The potential pollinators are attracted to the inflorescences where nectar may be produced in millilitre quantities. The flowers of *Embothrium lanceolatum* and *Grevillea robusta*, South American and Australian genera respectively of the Proteaceae, produce nectar which is largely composed of sucrose and the pollinators of these species are unknown (Per-

cival, 1961). Very little attention has been given to the quality and quantity of nectar produced by different species of *Protea*. This paper describes the production, carbohydrate composition and total nitrogen of floral nectar of a number of *Protea* species indigenous to the south-western Cape.

The mechanisms of nectar production are not fully understood although models have been suggested (Reed, Findlay and Mercer, 1971; Lüttge and Schnepf, 1976). As a first step in the understanding of *Protea* nectar physiology, the translocation of C-¹⁴ assimilates from fed leaves to nectar glands and bracts of inflorescences of *P. neriifolia* was undertaken.

MATERIAL AND METHODS

All the inflorescences were selected from *Protea* shrubs growing on the same aspect and under similar soil conditions at the National Botanic Gardens, Kirstenbosch, Cape Town. Only one population of a given species was sampled at 12h00–15h00 on sunny days at least 3 days after rains during April–August, 1978. The inflorescences were grouped into three age categories: new, half-new and old inflorescences.

1. New—a stage when the mature bracts are open revealing erect, densely packed and unopened florets.
2. Half-new—a stage when the florets are beginning to open in which the erect style becomes fully exposed functioning as the pollen presenter.
3. Old—a stage when all the florets in the inflorescences are completely open.

Nectar was removed from the inflorescences by means of a Pasteur pipette and samples were transferred to the laboratory and immediately frozen. The carbohydrate composition was identified and quantitatively determined by means of gas-liquid chromatography of their trimethylsilyl (TMS) derivatives using the methods of Holligan and Drew (1971), and Mitchell and Roberts (1973). Each sample for analysis contained 50 $\mu\ell$ nectar and 1 mg erythritol as the internal standard. Carbohydrates were characterised by comparison of their retention times relative to erythritol with those of standard compounds. TMS derivatives of standard carbohydrates were co-chromatographed to confirm the identity of individual peaks. A quantitative estimate was made by measuring the peak heights and referring to calibration curves of standard carbohydrates.

For total nitrogen determinations, 4 ml of three nectar samples of each species was analysed by means of the micro-Kjeldahl method.

Nectar volume in each inflorescence was estimated by initially collecting nectar using a Pasteur pipette and any residual nectar was removed by rinsing with 100 ml of distilled water. Any contamination of the diluted nectar by pollen and other solids during the rinsing process was kept to a minimum. Aliquots of pure nectar (0.5–0.2 ml) and diluted nectar (3.5 ml) were oven-dried at 80°C and

weighed. The total nectar volume in each inflorescence was then determined using the following formula:

$$\frac{100 \text{ (vol. pure nectar) (wt. dil. nectar)}}{\text{(vol. dil. nectar) (wt. pure nectar)}} + \frac{\text{orig. vol.}}{\text{pure nectar}}$$

C_{14} assimilation studies were undertaken by enclosing the foliose part of shoots of *P. neriifolia* containing one half-new inflorescence in a bell jar (300 × 250 mm). The base of the stem was immersed in a beaker of distilled water. The stem projecting the inflorescence through the top of the bell jar was sealed with Plasticine. An excess of 10 % lactic acid was added to 20 μ Ci of sodium C_{14} carbonate in a beaker in the bell jar to release $^{14}\text{CO}_2$. At the end of 30 minute incubation, the bell jar was removed to allow the shoots to undergo 24 hour "chase" period. For the duration of the experiment, the excised flowering shoots were maintained at 25 °C day and 20 °C night temperatures with an 8 hour day-length of 30 000 lux from fluorescent and tungsten lamps. Nectar was extracted during the day and night phases of incubation and at the end of 24 hour "chase" period the fed leaves and bracts were removed and plunged into boiling 80 % ethanol.

Aliquots of nectar (0.1 ml) were blended with 2 ml Beckman G.P. scintillation cocktail. Leaf and bract tissues were homogenised in 80 % ethanol using an Ultra-Turrax homogeniser. The ethanol-soluble extracts were filtered from the residue using Whatman no. 1 filter paper and both extracts were evaporated to dryness. The ethanol-soluble components were dissolved in distilled water and aliquots (0.1 ml) were blended with 2 ml Beckman G.P. scintillation cocktail. Portions of the insoluble components (100 mg) were transferred to scintillation vials and solubilised for 24 hours in 0.2 ml 60 % perchloric acid and 0.4 ml 30 % H_2O_2 using the method of Lobban (1974). After solubilisation, 5 ml of Packard Dimulume was added. Radioactivity was monitored on a Beckman L S 150 scintillation spectrometer for 20 minutes. All counts were converted to disintegrations per minute (DPM) after correcting for quenching using external standard ratio and C_{14} toluene.

RESULTS

Carbohydrate composition, nitrogen content and total volume of nectar

The sugar composition of the nectar consists of sucrose, glucose and fructose (Table 1). Fructose and glucose was detected in approximately equal proportions indicating that these reducing sugars are probably derived from enzymatic

TABLE 1.
Carbohydrate composition (mg ml⁻¹) of nectar of *Protea* spp.

		Fructose	Glucose	Sucrose
<i>P. repens</i> (L.) L.	New	55.5 ± 1.6	35.6 ± 0.9	—0
	Half-new	139.1 ± 28.7	110.1 ± 15.6	2.1 ± 2.1
	Old	27.7 ± 23.4	14.7 ± 11.9	2.3 ± 2.2
<i>P. nerifolia</i> (L.) L.	New	27.7 ± 13.4	21.3 ± 12.1	1.1 ± 0.7
	Half-new	44.1 ± 23.0	43.9 ± 22.1	1.6 ± 0.8
<i>P. sulphurea</i> Phill.	New	27.7 ± 8.0	61.9 ± 8.9	53.0 ± 7.3
	Half-new	62.5 ± 4.7	46.6 ± 5.2	77.3 ± 4.4
<i>P. tenax</i> (Salisb.) R.Br	New	63.1 ± 12.8	26.1 ± 10.8	60.4 ± 35.2
	Half-new	70.0 ± 10.6	61.2 ± 9.8	40.4 ± 11.4
<i>P. denticulata</i> Rourke	New	34.9 ± 4.6	31.9 ± 4.7	66.7 ± 16.9
	Half-new	29.9 ± 5.2	26.5 ± 5.2	9.4 ± 1.7
<i>P. longifolia</i> Andr.	Half-new	46.3 ± 16.1	128.5 ± 25.8	191.6 ± 77.7
	Old	68.8 ± 8.6	80.5 ± 31.3	128.3 ± 49.9
<i>P. humiflora</i> Andr.	Half-new	46.3 ± 4.6	31.9 ± 4.7	66.7 ± 16.9

Values are means of three separate analyses ± standard error of the mean.

hydrolysis of sucrose (Von Handel, Haeger and Hansen, 1972). There appears to be considerable interspecific variation of nectar composition. The sugar composition ranges from almost exclusively fructose-glucose (*P. repens* and *P. neriifolia*) to a balanced mixture of sucrose, glucose and fructose (*P. sulphurea*, *P. tenax*, *P. humiflora* and *P. denticulata*) to a sucrose dominating nectar (*P. longifolia*).

Only sufficient nectar was collected from *P. repens* and *P. neriifolia* for total nitrogen determinations. Total nitrogen content of *P. neriifolia* and *P. repens* was $46,7 \pm 11,7$ and $36,5 \pm 7,4 \mu\text{g ml}^{-1}$ respectively.

Total volume of nectar in the inflorescences is shown in Table 2 and *P. neriifolia* and *P. repens* produced the largest volume of nectar.

TABLE 2.
Volume of nectar (ml inflorescence⁻¹) produced in the inflorescences of *Protea* spp.

	New	Half-new	Old
<i>P. repens</i>	4,4 ± 0,6	4,9 ± 0,4	0,6 ± 0,2
<i>P. neriifolia</i>	5,2 ± 0,0	5,3 ± 0,0	6,5 ± 0,2
<i>P. sulphurea</i>	0,1 ± 0,1	1,3 ± 0,1	—
<i>P. denticulata</i>	—	0,6 ± 0,5	—
<i>P. tenax</i>	0,2 ± 0,1	0,4 ± 0,2	—
<i>P. longifolia</i>	—	0,9 ± 0,1	0,3 ± 0,0

Results are means of three separate determinations ± standard error of the mean.
(—) denotes no analysis.

Translocation of C₁₄ labelled assimilates to inflorescences of *P. neriifolia*

Figure 1 shows the radioactivity of nectar collected at intervals of time after exposure of fed leaves to ¹⁴CO₂. There was a 2 hour lag period and then radioactivity in the nectar increased to a maximum after 20 hours during the "chase" period. The accumulation of activity was not affected by the dark phase of incubation.

At the end of the 24 hour "chase" period, the fed leaves and bracts of the inflorescences were analysed for ethanol-soluble and -insoluble radioactive compounds. A significant proportion of C₁₄ labelled assimilates was translocated to the bracts and these assimilates were stored as insoluble compounds (Table 3).

DISCUSSION

Nectar of *Protea* species consists entirely of sucrose, glucose and fructose with nitrogenous compounds forming a minor component. C₁₄ assimilation studies of *P. neriifolia* have revealed that translocation of compounds to floral nectaries is

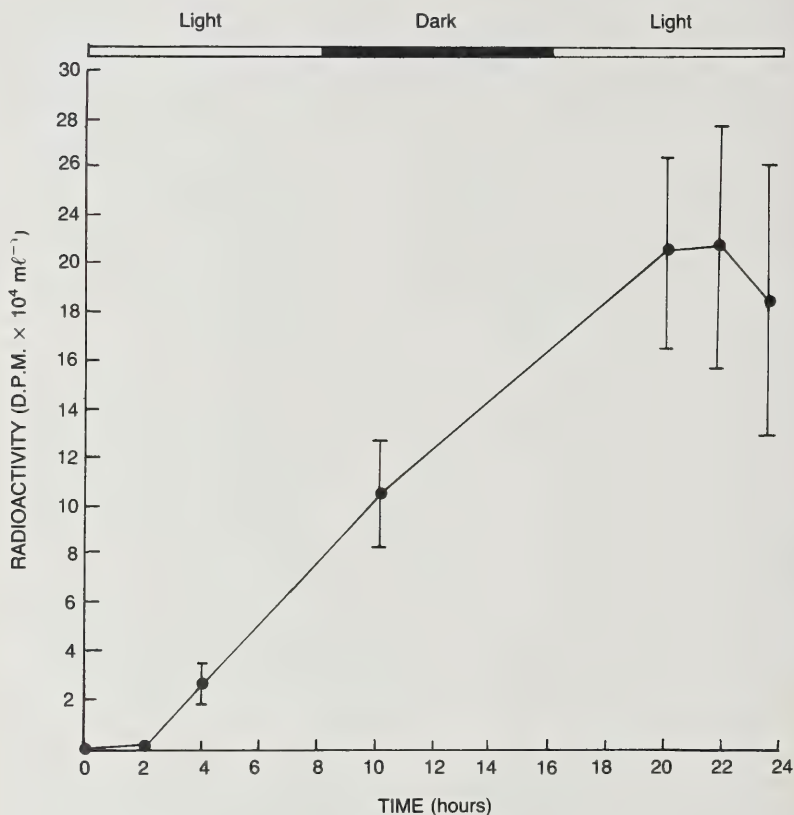


FIG. 1.

Radioactivity of nectar from inflorescences of *P. neriifolia* during the "chase" period after feeding leaves of shoots in $^{14}\text{CO}_2$ for 30 minutes.

not affected by dark incubation. The accumulated assimilates in the bracts may act as an alternative source of carbon which could then be re-directed to the floral nectaries.

There was however, a wide range of nectar types among *Protea* species in terms of the proportions of sucrose, fructose and glucose. The intraspecific constancy of sugar composition in *Protea* nectars is in agreement with other data (Wykes, 1952a; Percival, 1961; Van Handel *et al.*, 1972; Rowley, 1976). The

TABLE 3.

Recovered radioactivity in the fed leaves, bracts and floral nectar of *P. neriifolia* 24 hours after exposure to ¹⁴CO₂.

	Fed leaves	Bracts	Nectar
Total DPM × 10 ⁴	185,2 ± 42,3	21,5 ± 6,2	78,5 ± 6,4
Ethanol-soluble (%)	0,9	0,7	100
Ethanol-insoluble (%)	99,1	99,3	0

Results expressed as means of three separate feeding experiments

± standard error of the mean.

The shoots contained one half-new inflorescence and three leaves with a mean area of 75,0 ± 9,6 cm² leaf⁻¹.

results presented in this paper agree with those of Mostert *et al.*, (1980) who found that *P. repens* nectar consisted of reducing sugars, although G.L.C. analysis showed negligible amounts of sucrose compared with small but significant levels of this disaccharide in these studies.

Mostert *et al.* (1980) demonstrated that nectar of *P. repens* could be a major energy source for the Cape sugarbird. *P. repens* nectar was however low in protein and amino acids, but insects in the inflorescences may be the major source of nitrogen for the Cape sugarbird. In this study, *P. repens* and *P. neriifolia* produced the largest volume of nectar. Both of these species have large, brightly coloured terminal inflorescences, which are important features for bird pollination. The large, conspicuous and erect inflorescences of *P. longifolia* have green-coloured bracts and produce small quantities of sucrose-dominated nectar. The question arises whether the pollination strategy of *P. longifolia* is very different from that of related species.

The rodent-pollinated species appear to have small saucer-shaped dull coloured inflorescences and are borne at or near the ground (Rourke and Wiens, 1977). In this study, species which may be rodent-pollinated (e.g. *P. tenax* and *P. denticulata*) produce small volumes of nectar rich in sucrose. Bracts of *P. amplexicaulis* are known to be eaten by the rodent pollinators and thus may act as an alternative food source (Rourke, pers. comm.).

Direct evidence of pollinators preferring specific components in the nectar is very limited. Attraction by honeybees can be determined by the composition and concentration of nectar (Butler, 1945; Wykes, 1952b). A hierarchy of factors influencing taste and colour preferences has been demonstrated in hummingbirds (Stiles, 1976). Hummingbirds preferred solutions containing sucrose (Stiles, 1976), whereas starlings are attracted to glucose and fructose solutions but rejected sucrose (Schüler, 1978).

Preliminary "T" maze experiments using rodents indicate that test animals forage on *P. humiflora* almost exclusively when presented with a choice of it and *P. repens* (Wiens, pers. comm.). Until more information on pollination activity

in *Protea* is available, discussion on the possible links between nectar characteristics and pollination ecology remains largely speculative.

ACKNOWLEDGEMENTS

We thank Dr. John Rourke, Compton Herbarium, for helpful discussion and C.S.I.R., Atomic Energy Board and University of Cape Town for financial assistance.

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